FIG. 1A

Nucleotide sequence of EOS (SEQ.ID.NO1)

CCACGCGTCCGACCAGAGTCCAAGCCCTAGGCAGTGCCACCCTTACCCAGCCCAGCCTTG
AAGACAGAATGAGAGGGGTTTCCTGTCTCCAGGTCCTGCTCCTTCTGGTGCTGGGAGCTG
CTGGGACTCAGGGAAGGAAGTCTGCAGCCTGCGGGCAGCCCCGCATGTCCAGTCGGATCG
TTGGGGGCCGGGATGGCCGGGACGGAGAGTGGCCGTGGCAGGCGAGCATCCAGCATCCTG
GGGCACACGTGTGCGGGGGGTCGCTCATCGCCCCCCAGTGGGTGCTGACAGCGGCGCACT
GCTTCCCCAGGAGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGG
GCTCCACCTCGCCCCGCACGCTCTCGGTGCCCGTGCGACGGGTGCTGCTGCCCCCGGACT
ACTCCGAGGACGGGGCCCGCGGCGACCTGGCACTGCTGCAGCTGCGTCGCCCGGTGCCCC
TGAGCGCTCGCGTCCAACCCGTCTGCCTGCCCGTGCCCGGCGCCCGCCCGCCGCCCGGCA
CACCATGCCGGGTCACCGGCTGGGGCAGCCTCCGCCCAGGAGTGCCCCTCCCAGAGTGGC
GACCGCTACAAGGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACC
ACGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTCTGTGTGCCG
GCTACCCCCAGGGCCACAAGGACGCCTGCCAGGGTGATTCTGGGGGACCTCTGACCTGCC
TGCAGTCTGGGAGCTGGGTCCTGGTGGGCGTGGTGAGCTGGGGCAAGGGTTGTGCCCTGC
CCAACCGTCCAGGGGTCTACACCAGTGTGGCCACATATAGCCCCTGGATTCAGGCTCGCG
TCACTTCTAATGCTAGCCGGTGAGGCTGACCTGGAGCCAGCTGCTGGGGTCCCTCAGCCT
CCTGGTTCATCCAGGCACCTGCCTATACCCCACATCCCTTCTGCCTCGAGGCCAAGATGC
CTAAAAAAGCTAAAGGCCACCCCACCCCCCACCCACCACCTTCTGGCTCCTCTCCTCTTT
GGGGATCACCAGCTCTGACTCCACCAACCCTCATCCAGGAATCTGCCATGAGTCCCAGGG
AGTCACACTCCCCACTCCCTTCCTGGCTTGTATTTACTTTTCTTGGCCCTGGCCAGGGCT
GGGCGCAAGGCACGCAGTGATGGGCAAACCAATTGCTGCCCATCTGGCCTGTGTGCCCAT
CTTTTTCTGGAGAAAGTCAGATTCACAGCATGACAGAGATTTGACACCAGGGAGATCCTC
CATAGCTGGCTTTGAGGACACGGGGACCACAGCCATGAGCGGCCTCTAAGAGCTGAGAGA
CAGCCGGCAGGGAATCGGAACCCTCAGACCCACAGCCGCAAGGCACTGGATTCTGGCAGC
ACCCTGAAGGAGCTGGGAAGTAAGTTCTTCCCCAGCCTCCAGATAAGAGCCCCGCCGGCC
AATCCCTTCATTTCAACCTAAAGAGACCCTAAGCAGAGAACCTAGCTGAGCCACTCCTGA
CCTACAAAGTTGTGACTTAATAAATGTGTGCTTTAAGCTGCCAAAAAAAAAAA

FIG. 1B

Amino Acid sequence of EOS protease (SEQ.ID.NO.:7)

Met Arg Gly Val Ser Cys Leu Gln Val Leu Leu Leu
Leu Val Leu Gly Ala Ala Gly Thr Gln Gly Arg Lys
Ser Ala Ala Cys Gly Gln Pro Arg Met Ser Ser Arg
Ile Val Gly Gly Arg Asp Gly Arg Asp Gly Glu Trp
Pro Trp Gln Ala Ser Ile Gln His Pro Gly Ala His
Val Cys Gly Gly Ser Leu Ile Ala Pro Gln Trp Val
Leu Thr Ala Ala His Cys Phe Pro Arg Arg Ala Leu
Pro Ala Glu Tyr Arg Val Arg Leu Gly Ala Leu Arg
Leu Gly Ser Thr Ser Pro Arg Thr Leu Ser Val Pro
Val Arg Arg Val Leu Leu Pro Pro Asp Tyr Ser Glu
Asp Gly Ala Arg Gly Asp Leu Ala Leu Leu Gln Leu
Arg Arg Pro Val Pro Leu Ser Ala Arg Val Gln Pro
Val Cys Leu Pro Val Pro Gly Ala Arg Pro Pro Pro
Gly Thr Pro Cys Arg Val Thr Gly Trp Gly Ser Leu
Arg Pro Gly Val Pro Leu Pro Glu Trp Arg Pro Leu
Gln Gly Val Arg Val Pro Leu Leu Asp Ser Arg Thr
Cys Asp Gly Leu Tyr His Val Gly Ala Asp Val Pro
Gln Ala Glu Arg Ile Val Leu Pro Gly Ser Leu Cys
Ala Gly Tyr Pro Gln Gly His Lys Asp Ala Cys Gln
Gly Asp Ser Gly Gly Pro Leu Thr Cys Leu Gln Ser
Gly Ser Trp Val Leu Val Gly Val Val Ser Trp Gly
Lys Gly Cys Ala Leu Pro Asn Arg Pro Gly Val Tyr
Thr Ser Val Ala Thr Tyr Ser Pro Trp Ile Gln Ala
Arg Val Thr Ser Asn Ala Ser Arg

FIG. 2

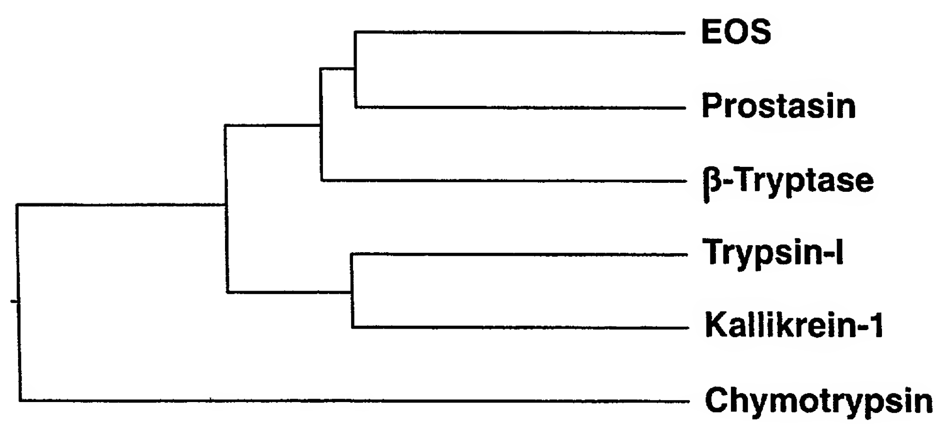

EOS
Prostasin
β-Tryptase
Trypsin-I
Kallikrein-1
Chymotrypsin

FIG. 3

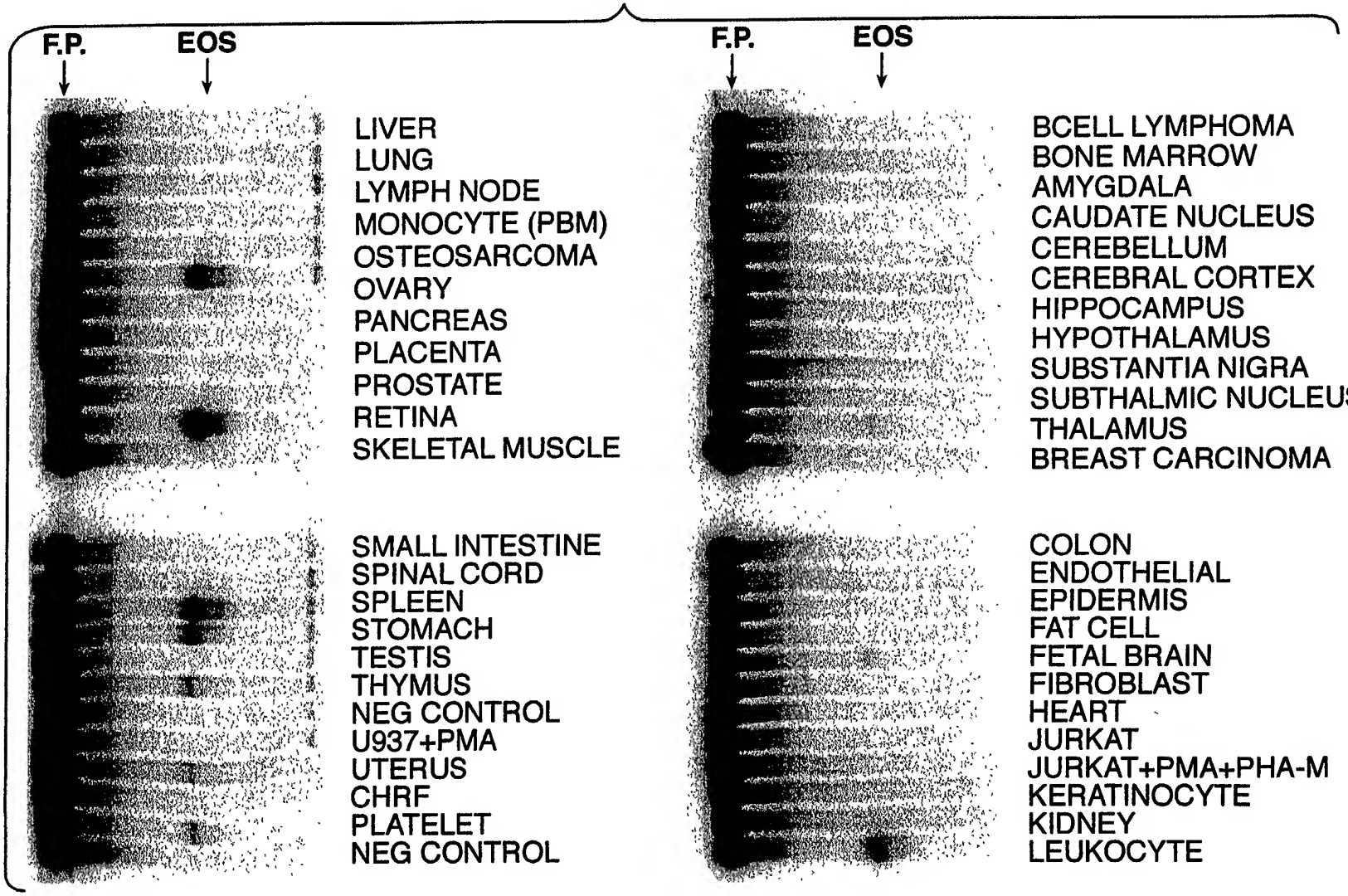

F.P.
EOS
LIVER
LUNG
LYMPH NODE
MONOCYTE (PBM)
OSTEOSARCOMA
OVARY
PANCREAS
PLACENTA
PROSTATE
RETINA
SKELETAL MUSCLE
SMALL INTESTINE
SPINAL CORD
SPLEEN
STOMACH
TESTIS
THYMUS
NEG CONTROL
U937+PMA
UTERUS
CHRF
PLATELET
NEG CONTROL
F.P.
EOS
BCELL LYMPHOMA
BONE MARROW
AMYGDALA
CAUDATE NUCLEUS
CEREBELLUM
CEREBRAL CORTEX
HIPPOCAMPUS
HYPOTHALAMUS
SUBSTANTIA NIGRA
SUBTHALMIC NUCLEUS
THALAMUS
BREAST CARCINOMA
COLON
ENDOTHELIAL
EPIDERMIS
FAT CELL
FETAL BRAIN
FIBROBLAST
HEART
JURKAT
JURKAT+PMA+PHA-M
KERATINOCYTE
KIDNEY
LEUKOCYTE

FIG. 4A

```
       Eco RI
       GAATTCACCACCATGGACAGCAAAGGTTCGTCGCAGAAATCCCGCCTGCT
  1    ------------------+------------------+------------------+------------------+------------------+   50
       CTTAAGTGGTGGTACCTGTCGTTTCCAAGCAGCGTCTTTAGGGCGGACGA
                   M   D   S   K   G   S   S   Q   K   S   R   L   L
                   |____________ Prolactin Signal Sequence ____________

       CCTGCTGCTGGTGGTGTCAAATCTACTCTTGTGCCAGGGTGTGGTCTCCG
 51    ------------------+------------------+------------------+------------------+------------------+   100
       GGACGACGACCACCACAGTTTAGATGAGAACACGGTCCCACACCAGAGGC
         L   L   L   V   V   S   N   L   L   L   C   Q   G   V   V   S
       ____________________ Prolactin Signal Sequence ______________|

                                                  Not I
       ACTACAAGGACGACGACGACGTGGACGCGGCCGCTCTTGCTGCCCCCTTT
101    ------------------+------------------+------------------+------------------+------------------+ 150
       TGATGTTCCTGCTGCTGCTGCACCTGCGCCGGCGAGAACGACGGGGGAAA
       D   Y   K   D   D   D   D   V | D   A | A   A   L   A   A   P   F
       ____ FLAG __________________|________|__________________ EK Pro ______

                                                      Xba I
       GATGATGATGACAAGATCGTTGGGGGCTATGCTCTAGAGGACGGAGAGTG
151    ------------------+------------------+------------------+------------------+------------------+   200
       CTACTACTACTGTTCTAGCAACCCCCGATACGAGATCTCCTGCCTCTCAC
       D   D   D   D   K   I   V   G   G   Y   A | L   E   D   G | E   W
       _______________ EK Pro ________________|______________|______________
```

FIG. 4B

```
      GCCGTGGCAGGCGAGCATCCAGCATCCTGGGGCACACGTGTGCGGGGGGT
201   ----------+---------+---------+---------+---------+ 250
      CGGCACCGTCCGCTCGTAGGTCGTAGGACCCCGTGTGCACACGCCCCCCA
        P  W  Q  A  S  I  Q  H  P  G  A  H  V  C  G  G

      ---------- Protease EOS.CDS ----------

      CGCTCATCGCCCCCCAGTGGGTGCTGACAGCGGCGCACTGCTTCCCCAGG
251   ----------+---------+---------+---------+---------+ 300
      GCGAGTAGCGGGGGGTCACCCACGACTGTCGCCGCGTGACGAAGGGGTCC
      S  L  I  A  P  Q  W  V  L  T  A  A  H  C  F  P  R

      ---------- Protease EOS.CDS ----------

      AGGGCACTGCCAGCTGAGTACCGCGTGCGCCTGGGGGCGCTGCGTCTGGG
301   ----------+---------+---------+---------+---------+ 350
      TCCCGTGACGGTCGACTCATGGCGCACGCGGACCCCCGCGACGCAGACCC
        R  A  L  P  A  E  Y  R  V  R  L  G  A  L  R  L  G

      ---------- Protease EOS.CDS ----------

      CTCCACCTCGCCCCGCACGCTCTCGGTGCCCGTGCGACGGGTGCTGCTGC
351   ----------+---------+---------+---------+---------+ 400
      GAGGTGGAGCGGGGCGTGCGAGAGCCACGGGCACGCTGCCCACGACGACG
        S  T  S  P  R  T  L  S  V  P  V  R  R  V  L  L

      ---------- Protease EOS.CDS ----------
```

FIG. 4C

```
     CCCCGGACTACTCCGAGGACGGGGCCCGCGGCGACCTGGCACTGCTGCAG
401  ----------+---------+---------+---------+---------+ 450
     GGGGCCTGATGAGGCTCCTGCCCCGGGCGCCGCTGGACCGTGACGACGTC
     P  P  D  Y  S  E  D  G  A  R  G  D  L  A  L  L  Q
```

Protease EOS.CDS

```
     CTGCGTCGCCCGGTGCCCCTGAGCGCTCGCGTCCAACCCGTCTGCCTGCC
451  ----------+---------+---------+---------+---------+ 500
     GACGCAGCGGGCCACGGGGACTCGCGAGCGCAGGTTGGGCAGACGGACGG
      L  R  R  P  V  P  L  S  A  R  V  Q  P  V  C  L  P
```

Protease EOS.CDS

```
     CGTGCCCGGCGCCCGCCCGCCGCCCGGCACACCATGCCGGGTCACCGGCT
501  ----------+---------+---------+---------+---------+ 550
     GCACGGGCCGCGGGCGGGCGGCGGGCCGTGTGGTACGGCCCAGTGGCCGA
        V  P  G  A  R  P  P  P  G  T  P  C  R  V  T  G
```

Protease EOS.CDS

```
     GGGGCAGCCTCCGCCCAGGAGTGCCCCTCCCAGAGTGGCGACCGCTACAA
551  ----------+---------+---------+---------+---------+ 600
     CCCCGTCGGAGGCGGGTCCTCACGGGGAGGGTCTCACCGCTGGCGATGTT
     W  G  S  L  R  P  G  V  P  L  P  E  W  R  P  L  Q
```

Protease EOS.CDS

FIG. 4D

```
      GGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTCTACCA
601   ---------+---------+---------+---------+---------+ 650
      CCTCATTCCCACGGCGACGACCTGAGCGCGTGGACGCTGCCGGAGATGGT
       G  V  R  V  P  L  L  D  S  R  T  C  D  G  L  Y  H

      ———————————— Protease EOS.CDS ————————————

      CGTGGGCGCGGACGTGCCCCAGGCTGAGCGCATTGTGCTGCCTGGGAGTC
651   ---------+---------+---------+---------+---------+ 700
      GCACCCGCGCCTGCACGGGGTCCGACTCGCGTAACACGACGGACCCTCAG
        V  G  A  D  V  P  Q  A  E  R  I  V  L  P  G  S

      ———————————— Protease EOS.CDS ————————————

      TGTGTGCCGGCTACCCCCAGGGCCACAAGGACGCCTGCCAGGGTGATTCT
701   ---------+---------+---------+---------+---------+ 750
      ACACACGGCCGATGGGGGTCCCGGTGTTCCTGCGGACGGTCCCACTAAGA
      L  C  A  G  Y  P  Q  G  H  K  D  A  C  Q  G  D  S

      ———————————— Protease EOS.CDS ————————————

      GGGGGACCTCTGACCTGCCTGCAGTCTGGGAGCTGGGTCCTGGTGGGCGT
751   ---------+---------+---------+---------+---------+ 800
      CCCCCTGGAGACTGGACGGACGTCAGACCCTCGACCCAGGACCACCCGCA
       G G P L T C L Q S G S W V L V G V

      ———————————— Protease EOS.CDS ————————————
```

FIG. 4E

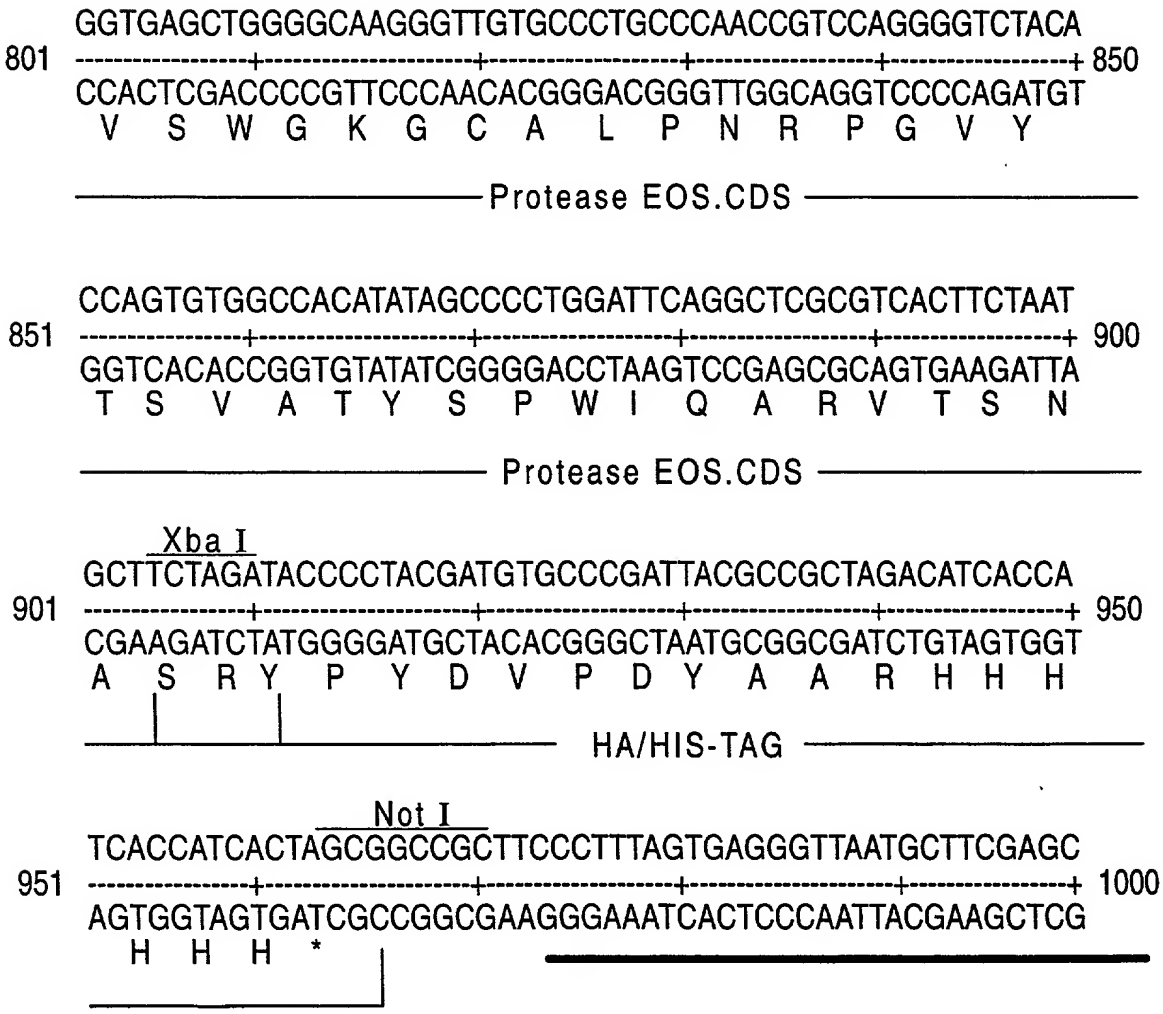


FIG. 4F

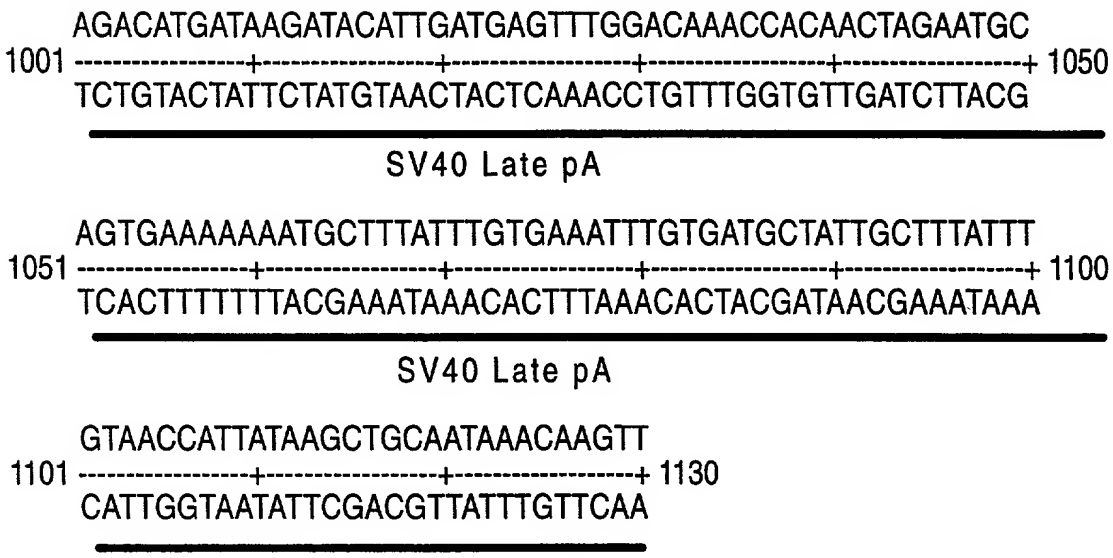
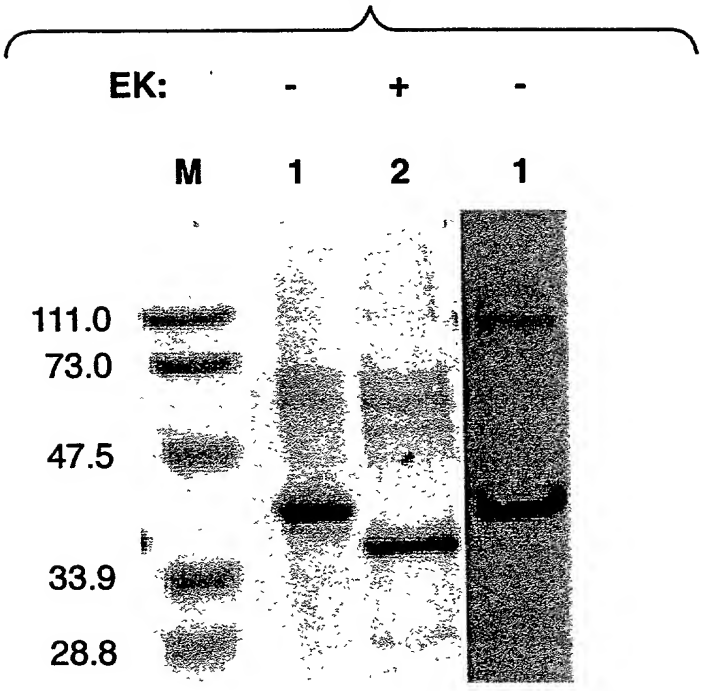


FIG. 5



Protease: PFEK2-EOS-6XHIS

FIG. 6

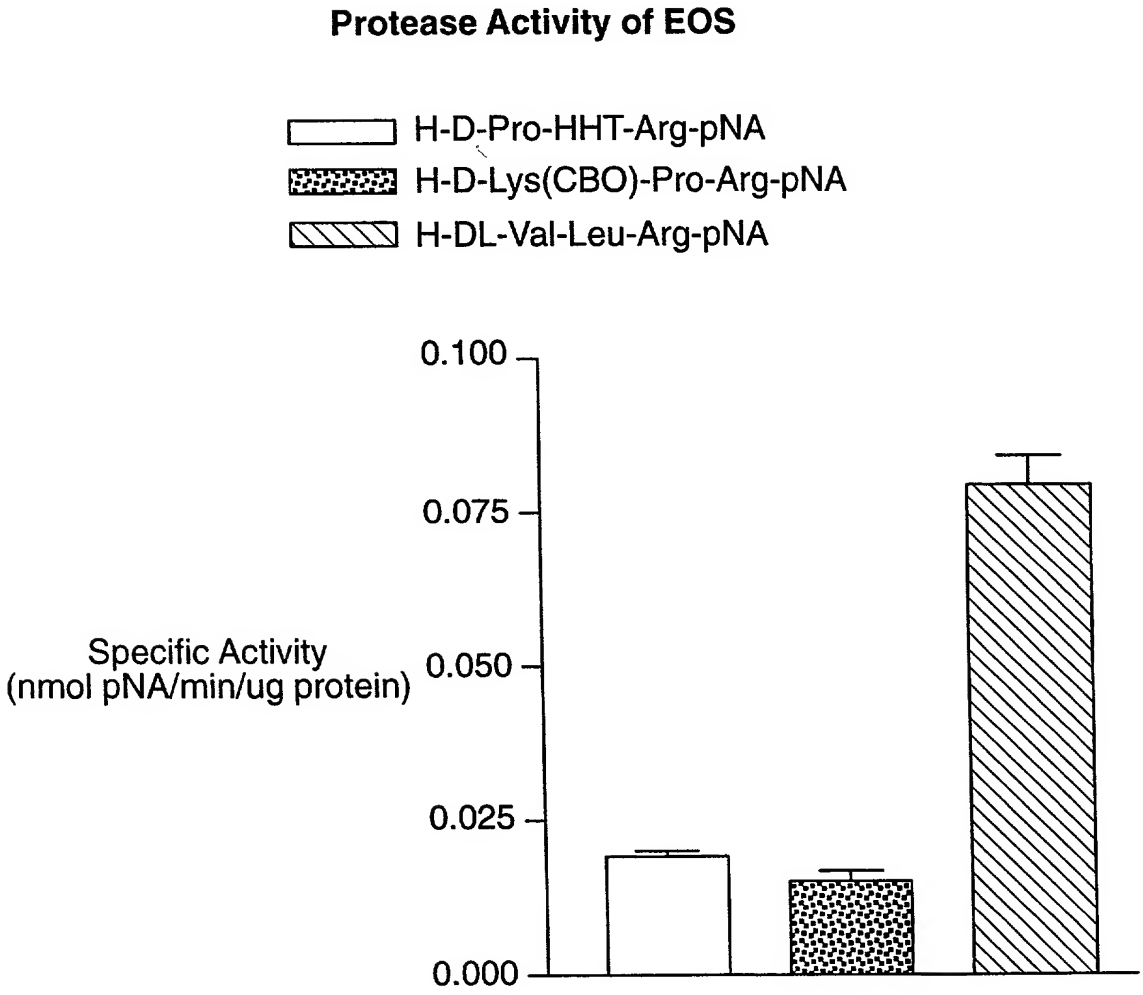

Protease Activity of EOS
H-D-Pro-HHT-Arg-pNA
H-D-Lys(CBO)-Pro-Arg-pNA
H-DL-Val-Leu-Arg-pNA
Specific Activity
(nmol pNA/min/ug protein)
0.100
0.075
0.050
0.025
0.000

FIG. 7

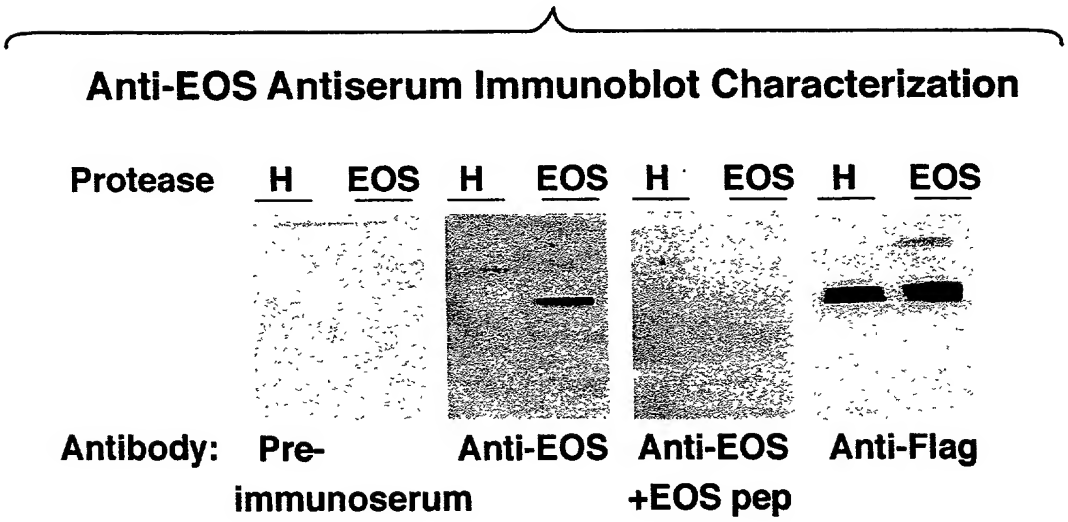

Anti-EOS Antiserum Immunoblot Characterization
Protease
H
EOS
H
EOS
H
EOS
H
EOS
Antibody:
Pre-immunoserum
Anti-EOS
Anti-EOS +EOS pep
Anti-Flag

FIG. 8

Localization of EOS protein (top) and mRNA (bottom) in human spleen, lung, and colon

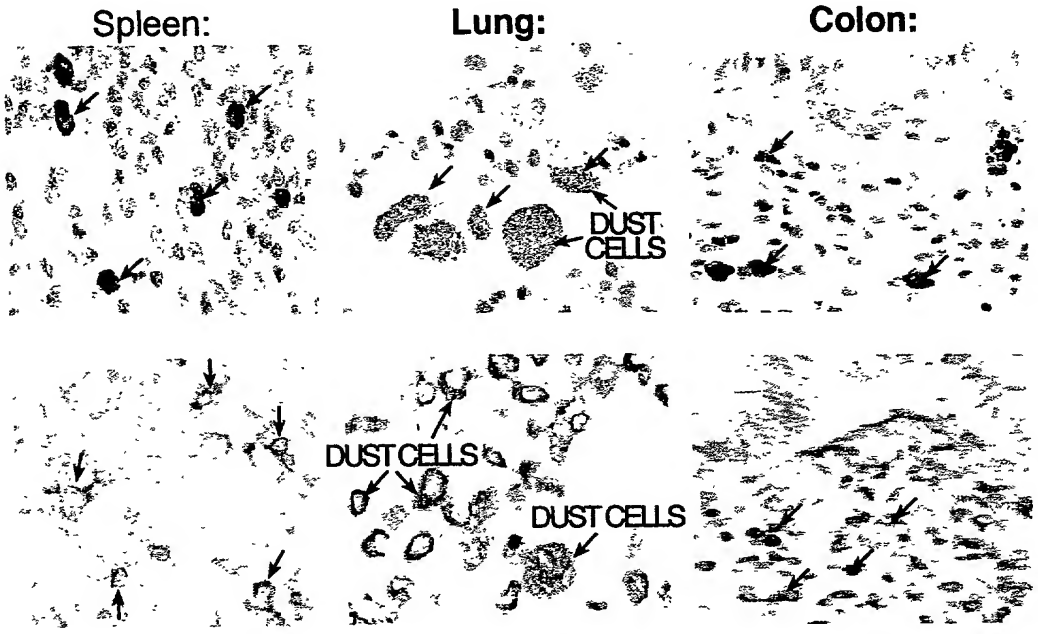
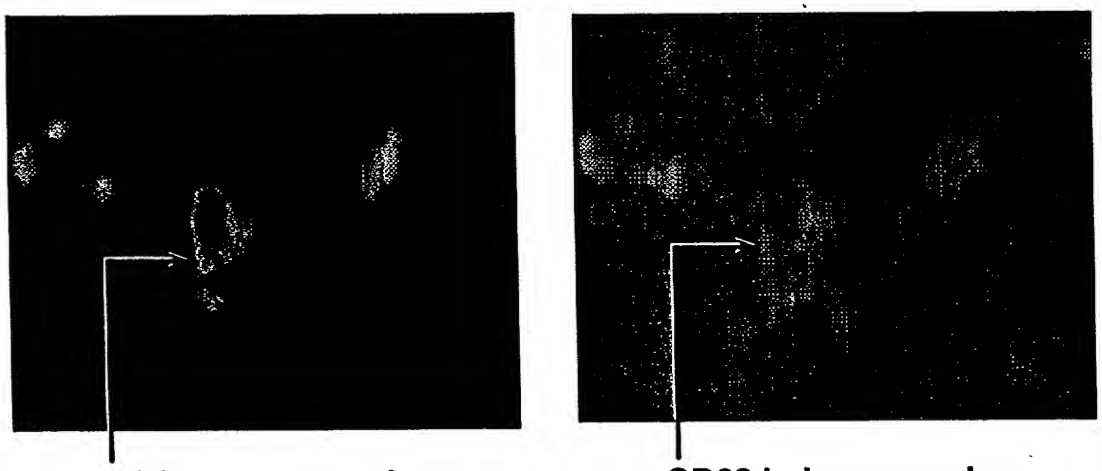


FIG. 9

Double immunofluorescencw (IF:IF) of EOS and macrophage marker:CD68



EOS in human colon

CD68 in human colon

FIG. 10

Up-regulation of EOS protein by PMA in U937 cells

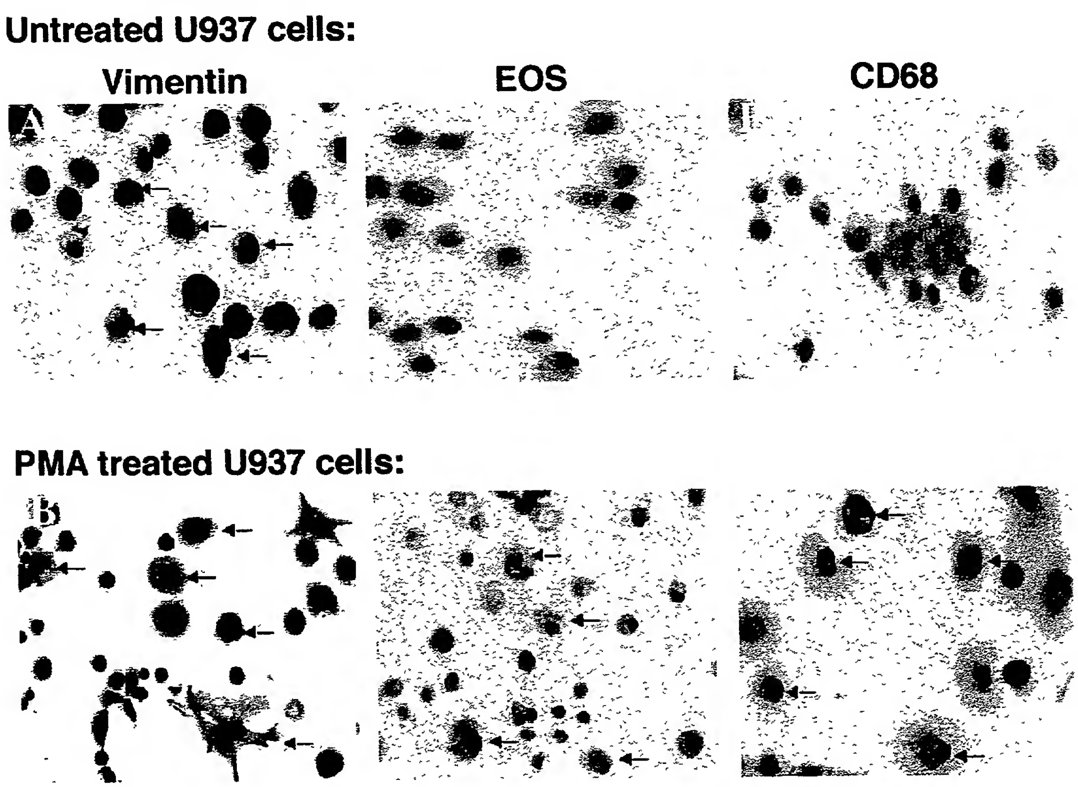


FIG. 11

Up-regulation of EOS mRNA by PMA in U937 cells